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# Stability of organic matter in forest soils of the middle Central Atlas: Extraction by biochemical fractionation

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## Abstract

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Organic matter is essential in forest ecosystems to maintain the structural stability of the soil. Following soil assimilation, it evolves in two opposite ways: mineralization and humification. This leads to the formation of a wide variety of substances that, evolve according to very different kinetics. This work involved studying the stability of organic matter in the soils of forest ecosystems: Atlas cedar, holm oak, zeen oak, and maritime pine. This study was performed on representative samples of the upper soil layer (0–30 cm) of the different ecosystems, using a biochemical fractionation technique. Results from the extraction of humic substances showed that humin is the main component (53 and 75%), followed by humic acids (19 and 34%) and fulvic acids (6 and 16%). These findings indicate stable carbon abundance and good sequestration of this element in the soils studied. Zeen oak and maritime pine have the most stable soil organic matter with a humification ratio of 4.30 and 4.10, respectively. Furthermore, the formation of humic substances is closely linked to the presence of clay that favors the formation of the clay-humic complex, making this material difficult to access by microorganism soils and therefore more resistant to decomposition. Ultimately, the studied soils have a stable organic matter which increases the dwell time of carbon in the soil. Such a result can be proved in further research, by using appropriate techniques like stable isotopes (<sup>13</sup>C).

## 1. Introduction

Organic matter in the soil component best reflects the interactions between the soil, the biosphere, and the atmosphere, because it depends both on the nature of the soil and the bioclimatic environment, which makes it an excellent indicator of the environment (Volkoff et al., 1988). Soil organic matter consists of molecules of different sizes which are more or less associated with each other and produced by the chemical transformation of bio-based substances (miscellaneous plant litter and animal) during humification (Borie, 2011). Organic matter transformations are carried out mainly by the processes of recombination (humification) and degradation (mineralization). The formation of stable products, "humus formation or humification", consists of recombinations and polymerizations of organic molecules, more or less complex. The biochemical nature of the organic matter brought to the soil determines the humification process (Borie, 2011). Complex humification of genesis mechanisms gen-

erates humic substances (Stevenson, 1994; Berthe, 2006), including, humin, humic acids (HA), and fulvic acids (FA), involving a variety of chemical reactions and biological transformation processes. Many factors that influence them include the origin and nature of the organic matter, the microorganisms involved in the decomposition process, and the microfauna as biological factors, as well as some physical parameters like pH, temperature, humidity, and soil aeration (Tahiri et al., 2014).

The evolution of humification over time is towards obtaining high molecular weights by increasing condensation. Fulvic acids (FA) are the lightest formed early process, followed by humic acids (HA). Humin is the latest step in humification and has a very high molecular weight (Robert, 2008).

Moroccan forest ecosystems, like Mediterranean forests, are composed of heterogeneous species, often clear and with diverse structures. These ecosystems generally meet their own nutrient needs through mineralization and organic matter storage in the soil. Humic substances, naturally present in a diffuse way in these

soils (Tahiri et al., 2014), influence their fertility by increasing biological activity, nutrient availability, and by complexing toxic metals (Eyheraguibel, 2004). Furthermore, these compounds improve soil structure, increase their stability, and therefore the time of their residence in the soil. This helps to reduce air emissions of CO<sub>2</sub> considered the main greenhouse gas (GHG). To our best knowledge, no study in Morocco has examined the capacity of forest soils to ensure the structural stability of organic matter. For this purpose, biochemical fractionation techniques of organic matter have been adopted to characterize the humic composition of this material for different sampling sites, having different forest vegetation and geological substrate characteristics. The main objective is to explore potential variations in the humic composition of organic matter in relation to these environmental characteristics, especially vegetation and geological substrate nature in the forest soils of the Middle Moroccan Central Atlas.

## 2. Materials and methods

### 2.1. Study area

The study was carried out at the level of Azrou, Jbel Aoua South, and Jaaba forests. The main dominant species are: Atlas cedar (*Cedrus atlantica*), holm oak (*Quercus rotundifolia*), zeen oak (*Quercus canariensis* Wild), and maritime pine (*Pinus pinaster* var. *maghrebiana*) of the mountain, with the presence of other secondary species (HCEFLCD, 2007).

The lithological scheme of the Middle Moroccan Central Atlas developed by Mhiyaoui (2016) summarizes the existing geological formations into primary formations (shales around

Tagounite), secondary formations represented by the Lower Jurassic (Lias) and very locally the Triassic (marls and red clays with dolerites or volcanic rocks) and quaternary formations (basaltic flows, volcanic ashes).

The soils represented in the study area mainly depend on the type of parent rock and are dominated by three classes: Cambisols, Nitisols-Alisols and liptosols according to World Reference Base for soil resources (WRB) classification of 2015 (El Mderssa et al. 2019).

The climate is cool and humid, with temperatures ranging from -3 to 2°C in January and reaching 31–37°C in July and about 830–982 mm of rainfall per year, based on 32 years of meteorological data (HCEFLCD, 2007).

### 2.2. Methodological approach

Taking into account the topo-climatic position and the type of stand, and with the help of a Geographic Information System (GIS) and field validation visits, nine (9) sites were selected that represent the different forest formations present in the study area (Table 1 and Fig. 1).

Precautions were taken to ensure that the physical and topographical conditions of the selected sites were similar in order to offset their effects in the analyses to be carried out.

At each site, soil samples from the first organo-mineral layer (<30 cm depth) were collected in three (3) replicates per site (Fig. 2). According to Benjelloun (1997), this layer has the highest concentration of roots and the most important exchanges between soil and plant roots.

After sieving the soil samples through 2 mm meshe, we proceeded to the extract humic substances, using the biochemical

**Table 1**  
Description of studied sites

Site n°	Forest	Coordinates	Exposition	Slope (%)	Height (m)	Parent rock	Stand forets
1	Jaaba	33°33'7"N -5°10'28"W	NW	14	1593	Basalt	A mixture of an adult forest of zeen oak and holm oak
2	Jaaba	33°33'16"N -5°10'38"W	N	10	1562	Basalt	Mature zeen oak forest
3	South Jbel Aoua	33°34'26"N -5°01'33"W	NE	13	1745	Sandy dolomite	Mature holm oak forest
4	South Jbel Aoua	33°34'21"N -5°01'26"W	NE	15	1750	Sandy dolomite	Young cedar forest
5	South Jbel Aoua	33°34'10"N -5°00'54"W	NE	18	1740	Sandy dolomite	Mature maritime pine forest
6	South Jbel Aoua	33°34'03"N -5°00'51"W	NE	20	1768	Sandy dolomite	Mixture of a young holm oak forest and a mature cedar forest
7	Azrou	33°29'57"N -5°08'31"W	NE	20	1690	Basalt	Pure cedar forest
8	Azrou	33°29'34"N -5°08'39"W	NE	10	1720	Basalt	Mature zeen oak forest
9	Azrou	33°29'30"N -5°08'43"W	NE	25	1710	Basalt	Mature holm oak forest

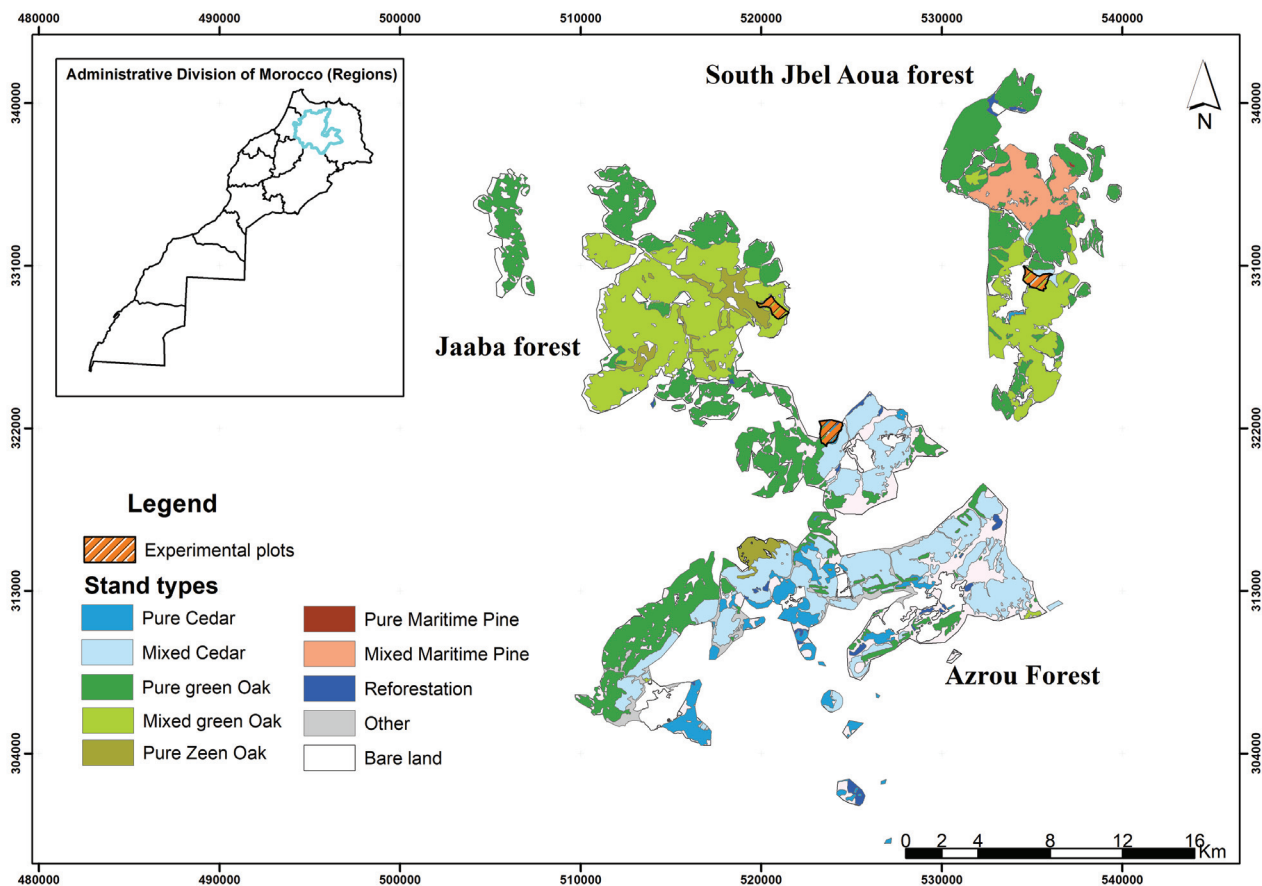


Fig 1. Map of the study area



Fig 2. Examples of plots studied and soil sampling by auger

fractionation process. The method adopted is the one recommended by the International Humic Substances Society (IHSS), cited by Serra-Wittling et al. (1996), which allows the extraction of humic acids and fulvic acids and their quantification by determining their carbon content. The relative carbon distribution in these different fractions is estimated to assess the degree of stability (resistance to mineralization) of the organic matter at the various sites analyzed, allowing them to be categorized using indicators such as:

- **CAH:** Carbon concentration in Humic Acids,
- **CAF:** Carbon concentration in Fulvic Acids,
- **CE:** Carbon concentration in the extractable fraction,
- **CT:** Total Carbon,
- **CH:** Carbon concentration in Humin.

#### Operative technique

Humic acids (HA) and fulvic acids (FA) are extracted by agitation for 2 hours on a rotary agitator of 2 g of the sample with 100 ml of 0.1M NaOH in 250 ml flasks. This alkali-soluble fraction (AH+AF) is recovered by centrifugation at 8627 g for 15 minutes and then filtered (Whatman GF-C glass fiber filter). The remaining quantity is then acidified to allow the precipitation of humic acids.

This acidification is performed on 20 ml of the supernatant with 1.8 ml of concentrated 1M H<sub>2</sub>SO<sub>4</sub> until a pH close to 1.5 (measured with a pH meter). After one night at +4°C, the acid-soluble fulvic fraction (AF) is separated from the insoluble humic fraction (AH) by centrifugation at 6320 g for 15 minutes and is collected after filtration (filter Whatman GF-C glass fiber filter).

**Table 2**  
Physicochemical properties of the studied soils

Site		(%) Clay	(%) C	(%) OM	(%) N	(%) C/N
S1	Min	14,3	2,74	4,72	0,21	7,33
	Max	48,4	8,18	14,1	0,41	24,9
	Mean ± SD	35,7 ± 11,39 a	4,97 ± 1,93 ab	8,58 ± 3,32 ab	0,30 ± 0,07 a	16,6 ± 7,30
S2	Min	14,4	2,42	4,18	0,01	2,50
	Max	48,2	14,7	25,4	2,46	298,5
	Mean ± SD	36,1 ± 11,4 a	6,26 ± 4,67 ab	10,8 ± 8,05 ab	0,60 ± 1,00 ab	10,5 ± 127
S3	Min	6,70	3,79	6,54	0,36	2,59
	Max	36,9	11,6	20,1	2,53	18,8
	Mean ± SD	29,2 ± 10,8 b	6,98 ± 2,77 a	12,0 ± 4,78 a	0,70 ± 0,78 ab	9,97 ± 6,90
S4	Min	8,40	2,64	4,54	0,18	2,70
	Max	38,9	9,81	16,9	0,98	56,1
	Mean ± SD	29,2 ± 13,3 ab	6,02 ± 2,66 ab	10,4 ± 4,60 ab	0,63 ± 0,30 ab	9,55 ± 20,4
S5	Min	6,30	1,26	2,18	0,01	2,71
	Max	40,5	10,2	17,5	0,86	129
	Mean ± SD	22,3 ± 13,4 c	5,12 ± 3,30 ab	8,82 ± 5,69 ab	0,52 ± 0,36 ab	9,84 ± 48,6
S6	Min	4,10	2,21	3,82	0,17	1,52
	Max	42,3	9,63	16,6	3,68	197
	Mean ± SD	29,9 ± 13,6 ab	4,68 ± 2,71 a	8,08 ± 4,68 a	0,20 ± 1,45 ab	23,5 ± 86,3
S7	Min	14,2	5,16	8,90	0,16	1,23
	Max	20,4	9,81	16,9	4,56	93,5
	Mean ± SD	17,1 ± 2,91 d	7,46 ± 2,20 ab	12,9 ± 3,79 ab	0,89 ± 1,86 ab	8,38 ± 42,9
S8	Min	18,10	5,48	9,45	0,02	2,24
	Max	36,40	18,4	31,6	4,76	874
	Mean ± SD	26,7 ± 6,76 ab	11,0 ± 4,55 b	18,9 ± 7,85 b	0,28 ± 1,85 ab	39,1 ± 342
S9	Min	18,9	7,48	12,9	0,77	2,06
	Max	36,4	16,2	27,9	4,45	9,91
	Mean ± SD	27,5 ± 6,71 ab	11,1 ± 3,50 b	19,1 ± 6,03 b	1,76 ± 1,31 b	6,30 ± 3,18

S1: stand of holm oak in mixture with zeen oak (Jaaba Forest), S2: stand of zeen oak (Jaaba Forest), S3: stand of holm oak (Jbel Aoua South Forest), S4: stand of cedar (Jbel Aoua South Forest), S5: stand of maritime pine (Jbel Aoua South Forest), S6: stand of cedar in mixture with holm oak, S7: Cedar stand (Azou Forest), S8: Zeen oak stand (Azrou Forest), S9: holm oak stand (Azrou Forest), Figures in brackets indicate coefficients of variation in %, similar letters mean that the values are not significantly different.

The measurement of carbon was assessed by the method of Walkley-Black (1934), and total nitrogen was carried out by the method of distillation Kjeldahl: Buchi 323 (1883). Organic matter (OM) content, was calculated as follows:  $MO (\%) = C (\%) * 1.724$  (Soltner, 1988). The Robinson pipette method was used to assess the particle size of the soils.

The analyses were carried out at the Laboratory of Environment and Valorization of Microbial and Plant Resources of the Faculty of Sciences of Meknes.

### 2.3. Statistical analysis

The statistical analysis of the data was conducted by ANOVA two ways (forest stand and geological substrate type), allowing the comparison of the means of the different variables. The hypotheses of this test, which are the normality of the distribution and the homogeneity of the variance, were previously verified by the Shapiro wilk and the Levene test at a 5% significance level respectively. These analyses were conducted using IBM SPSS version 25 software.

## 3. Results

### 3.1. Basic soil characteristics

Physicochemical properties of the soils in the studied sites (El Mderssa et al., 2019) show that most of the forest soils have a fairly coarse texture due to the lithological nature of the parent rock. The average organic carbon content varies from 4.69% (S6 site) to 11.10% (S9 site), and the distribution of organic carbon is relatively heterogeneous at all sites with a coefficient of variation of 29 to 74%. With regard to fertility, the recorded values showed that all sites are considered to have an extremely high total nitrogen content, according to the standard found by Dabin (1963) ranging from 0.15 to 0.25%.

### 3.2. Characterization of the humic composition

The results of the biochemical fractionation of the studied soils are given in Table 3. Three fractions are highlighted: humin, humic acids (HA), and fulvic acids (FA). In fact, humin fraction

Table 3

Distribution of organic carbon in the form of fulvic acids, humic acids, and humin expressed in % of total organic carbon

Site		C <sub>AF</sub> (%)	C <sub>AH</sub> (%)	C <sub>Humin</sub> (%)	C <sub>extractible</sub> /C <sub>total</sub> (%)	C <sub>AH</sub> /C <sub>AF</sub>
S1	Min	3,17	12,7	33,0	15,8	1,25
	Max	29,8	47,2	84,2	67,0	4,00
	Mean ± SD	16,1 ± 8,82 b	30,7 ± 12,6 ab	53,2 ± 19,1 a	46,78 ± 19,07 ab	2,36 ± 1,17 b
S2	Min	3,52	6,1	36,0	12,1	0,40
	Max	26,4	53,3	87,87	64,1	5,00
	Mean ± SD	12,9 ± 8,36 a	23,1 ± 17,9 ab	64,00 ± 19,91 a	36,00 ± 19,91 ab	2,23 ± 1,65 ab
S3	Min	2,23	19,2	45,4	26,7	1,00
	Max	26,7	35,6	73,3	54,6	11,00
	Mean ± SD	14,8 ± 8,32 ab	27,5 ± 6,25 ab	57,7 ± 11,2a	42,30 ± 11,22 ab	3,31 ± 3,82 a
S4	Min	5,23	26,13	25,9	31,35	1,60
	Max	28,5	45,58	68,7	74,07	7,00
	Mean ± SD	11,1 ± 8,69 b	34,39 ± 8,37 ab	54,5 ± 15,4 a	45,49 ± 15,42 ab	3,98 ± 1,85 b
S5	Min	3,65	26,3	38,6	32,9	2,00
	Max	20,5	44,1	67,1	61,4	8,00
	Mean ± SD	10,4 ± 6,07 a	33,23 ± 7,4 ab	56,4 ± 12,5 a	43,6 ± 12,5 ab	4,10 ± 2,25 ab
S6	Min	1,61	5,38	41,5	6,99	2,00
	Max	10,2	49,1	93,0	58,47	5,25
	Mean ± SD	5,64 ± 3,44 b	19,1 ± 16,4 ab	75,3 ± 19,4 a	24,73 ± 19,39 ab	3,23 ± 1,22 b
S7	Min	4,63	15,8	53,5	29,02	1,00
	Max	20,4	27,8	71,0	46,51	6,00
	Mean ± SD	14,6 ± 5,47 ab	23,0 ± 5,55 ab	62,4 ± 7,43 a	37,63 ± 7,43 ab	2,10 ± 1,92 a
S8	Min	4,23	14,1	48,1	18,3	3,00
	Max	9,69	42,5	81,7	52,0	7,00
	Mean ± SD	7,01 ± 2,40 b	29,2 ± 10,1 ab	63,78 ± 11,88 a	36,21 ± 11,88 ab	4,30 ± 1,43 b
S9	Min	3,96	13,8	58,49	17,8	0,50
	Max	27,7	30,7	82,19	41,5	3,50
	Mean ± SD	11,4 ± 8,73 ab	19,7 ± 6,32 ab	69,0 ± 9,79 a	31,0 ± 9,79 ab	2,44 ± 1,16 a

CAF: Fulvic acids, CAH: Humic acids, CHumine: Humine, Ctotal: Total organic carbon

S1 : holm oak stand mixed with zeen oak (Jaaba Forest), S2 : Zeen oak stand (Jaaba Forest), S3 : holm oak stand (Jbel Aoua South Forest), S4 : Cedar stand (Jbel Aoua South Forest), S5 : Maritime pine stand (Jbel Aoua South Forest), S6 : Cedar stand mixed with holm oak, S7 : Cedar stand (Azou Forest), S8: Zeen oak stand (Azrou Forest), S9: holm oak stand (Azrou Forest), Figures in brackets indicate coefficients of variation in %, similar letters mean that the values are not significantly different.

predominates in all soils studied. The observed value ranges from 53.21 to 75.26%. The highest value was found in a mixed layer of cedar and holm oak on sandy dolomite (S6 site).

The extractible or alkali-soluble fraction that corresponds to a brown flaky precipitate (humic acid) and a soluble supernatant (fulvic acid) was inversely proportional to the non-extractable carbon contained in humin. The highest values were observed in the cedar stands (S4), followed by the deciduous holm oak and zeen oak stands (S1), (S3), and (S2), with respectively: 54.49%, 46.79%, 42.30%, and 36%. These sites show blackish-colored humic acids, which indicate a high carbon concentration. The S6 site corresponding to the cedar-oak mixture had the lowest extraction carbon value (24.73%). This is due to the low value of fulvic acid (5.65%).

The CAH/CAF ratio varies from 2.11 (S7 site) to 4.30 (S8 site). According to Serra-Wittling (1996), this ratio is close to 0 for raw materials and reaches values between 2 and 5 for highly humified materials. All sites have humified organic matter make it clear that

fulvic acids are very mobile and quickly carried away by infiltration water, whereas humic acids are not very mobile (Calvet, 2003). Hence, the justification of the high values of the CAH /CAF ratio.

Results from the two-way ANOVA tests indicated that:

- Geological substrate has no significant effect on this humic composition ( $p > 0,05$ ).
- There is a significant difference between humin means and extractable fraction depending on vegetation type ( $p < 0,05$ ).
- The vegetation type does not significantly affect the difference in the means of the humification ratio ( $p > 0,05$ ).
- The interaction between geological substrate type and vegetation type is not significant, meaning that this interaction has no significant influence on the humic composition of the soil organic matter ( $p > 0,05$ ).

Pearson's correlation between organic matter stability and soil physicochemical properties shown in Table 4, revealed that CAH/CAF ratio had a positive and highly significant correlation with clay.

**Table 4**

Correlation matrix between the CAH/CAF humification ratio and the properties of the studied soils

	$C_{AH}/C_{AF}$	OM	C/N	CLAY
$C_{AH}/C_{AF}$	1	0,66**	0,52*	0,74**
OM		1	0,47*	0,87**
C/N			1	0,40
CLAY				1

\*  $p < 0.05$ ; \*\*  $p < 0.01$ 

#### 4. Discussion

##### 4.1. Influence of geological substrate on humic composition

The effect of the geological substrate nature can indirectly affect the humic composition of the soil organic matter. Indeed, the dolomite where the trees grow at sites S4 and S5, for example, is a sedimentary rock rich in calcium and magnesium, leading to an increase in the content of humic and fulvic acids (Hsieh et al., 2006). Sand dolomite also contains sand particles, which can affect the soil structure and its ability to hold water, another factor that can affect this composition (Wang et al., 2021). The chemical composition also of dolomite can influence the composition of humic substances in humus by providing nutrients to microorganisms involved in the decomposition of organic matter. The non-significant effect of geological substrate type that was shown by the Anova 2 test is explained by the basic environment characterizing the two geological substrates studied (basalt and dolomite). In fact, a study carried out by Aranda et al. (2014) showed that there is a significant difference on the humic composition of the organic matter of mediterranean forest soils developed on a basic and acidic lithological material. Thus, it seems that the nature of the parent material is not the main factor influencing the composition of humic substances. Indeed, in tropical forests, many authors associated humic composition to litter quality and the composition of soil macrofauna rather than to edaphic properties (Mabicka Obame Rolf et al. 2022). Other environmental factors can influence this composition, including type of vegetation present and the physicochemical properties of the soil (Aranda et al. 2014).

##### 4.2. Influence of the vegetation type on humic composition

According to Gladys (2001), the amount and chemical quality of litter produced by forest stands are among the environmental factors that determine the composition of humic substances in organic matter. The same author adds that edaphic factors are less important. Thus, the richness of humin in the soil under the mixed stand of cedar and holm oak (S6) is explained, by the presence of significant organic matter brought to the soil due to the high density of the holm oak stand in this forest, exceeding 640 stems/ha (El Mderssa et al., 2019). This allows to create favorable conditions for the humification (Aranda et al. 2014). The closure of the ecosystem prevents the penetration of solar radiation, which promotes humification that prevails over mineralization (Abril et al., 2013). This richness is also due to

the acidifying character of the litter beneath this resinous cedar-based ecosystem, where the chemical composition of the needles is rich in lignin. The latter decomposes slowly and is a recalcitrant fraction in soil (Aranda et al. 2014). Indeed, lignin is formed by a highly stable aromatic ring that is exceptional in the living world (Robert, 2008). These rings are linked together by more than 12 possible bonds (C-O and C-C), which are very stable to form an irregular three-dimensional network hardly degradable (Abril et al., 2013). Lignins are the main constituents of softwoods and their biopolymerization occurs in plant cell walls by enzymatic oxidation. They are present in needles, stems (up to 10% of the mass), and wood (up to 30% of the mass) (Reid, 1995). Microbial degradation of these compounds is carried out exclusively by fungi, the most efficient of which is white rot (Duchaufour, 1997). The solubility of the extractable fraction (humic acid and fulvic acid) in an alkaline medium is explained by the weaker organo-mineral bonds that can be easily broken during extraction. This solubility also results from the richness of the chemical compounds easily degradable by microorganisms, especially simple sugars called monosaccharides or oses, carbohydrates, and lipids. Proteins and hemicelluloses are also compounds of organic acids that are easily degradable (Duchaufour, 1997; Cédric, 2004). They are plant constituents that accompany cellulose in the constitution of wood. Following the data obtained, with regard to the forest ecosystems studied, soil organic matter is strongly linked to mineral particles rather than the extractable fraction which is less important. This is in agreement with the results found by Ndira (2006) in some forest ecosystems in France where the extracted organic carbon in relation to the total carbon represents 13.42% for Black pine (*Pinus nigra*); 16.11% for Pubescent oak (*Quercus pubescens*) and 23.46% for Eucalyptus (*Eucalyptus sp.*).

Despite their solubility in an alkaline medium, humic acids and fulvic acids present a different degree of humification. Indeed, fulvic acids are the simplest and have a low molecular weight, while humic acids are more condensed (Robert, 2008). Consequently, the ratio of the humic fraction to the fulvic fraction (CAH/CAF) is commonly used to evaluate the degree of condensation of extractable compounds (Saviozzi et al., 1988). The higher this ratio, the more polymerization takes place (Ndira, 2006).

The difference observed between the sites may be due to the difference in the density of the vegetation cover and the quality and quantity of organic matter brought to the soil. According to Duchaufour (1970), a high density leads a very strong humification and polymerization (grey humic acids are often much more abundant), allowing the formation of thick humus horizons.

Indeed, the ecosystems of zeen oak on basalt (S8), which show the highest humification ratios, are characterized by a very high stand density, reaching 186 stems/ha (El Mdersa, 2019), giving rise to a thick layer of litter. These results confirm those of several authors who showed that the composition of plant litter greatly influences SOM stability (Kovaleva and Kovalev, 2009; Poirier et al., 2003; Abril et al., 2013). These findings highlight the role of the tree layer in the humification and stability of organic matter, and subsequently in the accumulation of carbon stock.

Climatic variation, associated with seasonal alternations, also influences the degree of condensation of humic acids. According to Bachelier (1983), fulvic acids are more abundant in spring and summer (as a result of greater biological activity), and less abundant in autumn and winter, whereas humic acids behave in the opposite way to fulvic acids. Previous studies, especially the one carried out by Ndira (2006), have revealed that the CAH/CAF ratio records values of 1.51 for Pubescent Oak (*Quercus pubescens*); 1.91 for Eucalyptus (*Eucalyptus sp*), and 4.01 for Black Pine (*Pinus nigra*). All of these results show that the species, climate, and physicochemical conditions influence the formation of humic substances and their composition in the soil.

Pearson matrix indicates a positive correlation between the physicochemical properties of the soils studied and the organic humification process. This is due to the unique characteristics of clays, such as their small size, sheet-like structure, and negative charge, which allows them to form complex colloidal solutions with humic acids. When cations neutralize the surface charges of these particles, a cloud of positive charges consisting of hydrogen ions, metallic cations, or ammonia is formed around them, providing the soil with significant chemical fertility. Moreover, the formation of clay-humus complexes results in the aggregation of other soil particles, such as silts and sands, which ensures the structural longevity of the soil through microporosity and improves physical stability against external disturbances (Brady et al. 2016)

The correlation between the CAH/CAF ratio with organic matter and the C/N ratio is also positive and significant. The higher the soil organic matter, the greater the degree of humification. Indeed, the rate of extraction of humic substances is inversely proportional to the total carbon content and the C/N ratio (Ndira, 2006). A low C/N soil favors net mineralization, while a high C/N soil favors net immobilization (Dommergues and Mangenot, 1970). Lovett et al. (2002) demonstrated that the C/N ratio influences decomposition rates, and is associated with variations in plant composition.

## 5. Conclusions

In conclusion, our findings revealed that in the sites studied, the type of vegetation showed a significant effect on the humic composition of the soil's organic matter. However, for the nature of the geological substrate, except for the significant relationship between clay and humification ratio, no significant difference was detected. In addition to that, results of biochemical fractionation of the soil organic matter studied revealed that

humic is the most dominant fraction. These findings are due to the abundance of organic matter into a litter form, in particular associated with the density and phenology of the species present, the presence of hardly biodegradable chemical compounds, and also by the climatic factors of the central Middle Atlas region, especially the cold, which inhibit the mineralization process through the promotion of humification. Indeed, humin represents the soil's stable carbon. Its abundance indicates that carbon is well sequestered in the studied ecosystems. This characterization of humic substances in the forest soils of the Middle Atlas region has highlighted the important role they play in the stability of organic matter and the reduction of GHG emissions, especially CO<sub>2</sub>.

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